Analysis of the Nonpolar Extractables of *Asclepias speciosa*[1]

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ABSTRACT

Asclepias speciosa is currently being evaluated as a potential hydrocarbon-producing crop. Chemical analyses of the nonpolar extractables of the above ground parts of this plant showed that pentacyclic triterpenoids and sterols account for over 90% of the refined hexane extract. In addition, low molecular weight natural rubber (*cis*-1,4-polyisoprene), of potential use as an elastomer, was obtained from the crude hexane extract.

INTRODUCTION

There is considerable interest in latex-producing plants as renewable sources of chemical feedstocks (1–11). Many species in the milkweed family (Asclepiadaceae) are known to produce copious amounts of latex, and there has recently been increasing interest in the nonpolar constituents of these latex-bearing plants as potential new industrial raw materials (1–3, 6, 7, 11–14). Plants in the milkweed genera *Calotropis* (7, 12–14), and *Asclepias* (1–3, 6, 7, 11) have been identified as candidates for domestication as hydrocarbon-producing crops and are currently under investigation by various groups throughout the world.

Asclepias syriaca L. (the common milkweed) has recently been evaluated by Buchanan and coworkers as a potential hydrocarbon-producing crop for the central and eastern USA (1–3, 6). We are currently evaluating a closely related species, *A. speciosa* Torr. (the showy milkweed), as a potential source of chemical feedstocks to be grown on western semiarid lands. We have undertaken detailed analyses of sequential hexane and methanol extracts of *A. speciosa* by means of thin layer chromatography (TLC), glass capillary gas liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS) (electron impact [EI] and chemical ionization [CI]), high pressure liquid chromatography (HPLC) and gel permeation chromatography (GPC). In this report, we present the results of our investigation of the hexane extractables of *A. speciosa*. Although Buchanan and coworkers succeeded in fractionating the nonpolar extractables of *A. syriaca* into general lipid classes and in obtaining semiquantitative data based on TLC scanning densitometry (3, 6), the present work on *A. speciosa* constitutes the first detailed report on the composition of the nonpolar extractables from an *Asclepias* species seriously being considered for domestication and large-scale cultivation.

EXPERIMENTAL PROCEDURES

Extraction of Plant Material

Aerial parts of *A. speciosa* in full flower were collected on July 2, 1981 from both cultivated plots and wild stands near Salt Lake City, Utah. The plants were oven-dried at 70 C, ground in a Wiley Mill, and subsequently extracted with hexane in Soxhlet extractors for 20 hr. The solvent was then removed under reduced pressure to yield dark green semisolid hexane extracts (15).

Processing and Chemical Characterization of the Hexane Extracts

Pigments were removed from the crude hexane extracts by decolorizing with activated charcoal according to the procedure of Buchanan and coworkers (3), and natural rubber (*cis*-1,4-polyisoprene) was precipitated by the addition of acetone followed by centrifugation. Removal of the solvent in vacuo afforded yellow-orange hard waxes. These decolorized, rubber-free hexane extracts were then subjected to analysis by TLC, glass capillary GLC and GC-MS.

Analytical and preparative TLC were performed on 20 × 20 cm prescored silica gel GHLF plates (Analtech, Inc.; 0.25 mm) using hexane/ethyl ether/acetic acid (80:20:1) (3, 6) and methylene chloride/methanol/acetic acid (90:10:1) as solvent systems. Visualization for analytical TLC was accomplished under long- and shortwave UV light, followed by spraying with 50% aqueous H_2SO_4 and oven-heating at 120 C for 15 min. For preparative TLC, visualization using aqueous acid and heat was performed only on 5 × 20 cm sections of the TLC plates which were separated following development. Kedde reagent (16) was used for the specific detection of cardiac glycosides by TLC.

GLC analyses were performed with a Varian 1800 gas chromatograph equipped with a flame ionization detector (350 C) using a J & W DB-1 fused quartz capillary column (30 m × 0.32 mm id) with nitrogen as the carrier gas (18 cm/sec). All GLC analyses were performed in the split mode (25:1 split ratio) with the injector temperature at 275 C. The oven temperature was programmed from 120 to 350 C at 4 C/min, and peak areas were calculated using a Columbia Scientific Industries Supergrator-2 electronic digital integrator. All samples subjected to GLC and GC-MS analysis were dissolved in Tri-Sil 'Z' (Pierce Chemical Co.) to ensure complete silylation of all free hydroxyl and carboxyl groups.

GC-MS analyses were performed with a Finnigan Model 4000 Quadrupole Gas Chromatograph-Mass Spectrometer, taking mass spectral scans from mass 40 to mass 995 every 2 sec. Chromatographic separations were achieved using a fused silica capillary column (30 m × 0.25 mm id) coated with OV-101. All MS analyses were made in the splitless mode using He as carrier gas (40 cm/sec). The column temperature was ballistically heated from 50 to 120 C and then programmed to 300 C at 4 C/min. Compounds were identified by both EI (electron impact) and CI (chemical ionization) mass spectrometry (using methane as the ionizing gas) and by their order of elution and relative GLC retention times (17–24). The identities of all of the major components and several of the minor ones were confirmed by cochromatography (TLC, GLC) using authentic standards (Table II).

60 MHz proton NMR spectra of the acetone-precipitated rubber were obtained on a Varian EM-360 NMR spectrom-

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TABLE I

Analysis of the Major Fractions of the Hexane Extract of *A. speciosa*

Extract/compound class	% of the hexane extract	% of plant[a]
Total crude hexane extract	–	3.8
Pigments[b]	11.6 ± 1.3	0.4
Natural rubber[c,d]	2.2 ± 0.8	0.1
Remaining hexane extractables (refined hexane extract)[e]	86.3 ± 2.4	3.3

[a]Aerial parts; calculated on a dry weight basis (typical values).

[b]Determined by recovery after decolorization of the hexane extract with activated charcoal.

[c]Obtained by precipitation with acetone followed by centrifugation.

[d]Weight average molecular weight, M_W = 52,000; number average molecular weight, M_n = 32,000; polydispersity, M_W/M_n = 1.63.

[e]After removal of pigments and natural rubber.

TABLE II

Analysis of the Refined Hexane Extract (minus pigments and natural rubber) of *A. speciosa* by TLC, GLC, and GC-MS

Compound	R_f[a] A	R_f[a] B	RR_t[b]	%
Palmitoleic acid[d]	–	–	0.52	tr[c]
Palmitic acid[d]	–	–	0.54	tr
Linolenic acid methyl ester	–	–	0.63	tr
Margaric (heptadecanoic) acid[d]	–	–	0.64	tr
Phytol[d]	–	–	0.64	tr
Linolenic acid[d]	–	–	0.65	1
Stearic acid[d]	–	–	0.67	tr
Glyceryl-1-mono-palmitate[d]	–	–	0.90	tr
$C_{27}H_{56}$ alkane (heptacosane)	–	–	0.95	tr
Glyceryl-2-mono-linolenate[d]	–	–	0.99	tr
Squalene ($C_{30}H_{50}$)	0.89	0.92	1.00	tr
$C_{29}H_{60}$ alkane (nonacosane)	–	–	1.05	1
$C_{30}H_{62}$ alkane (triacontane)	–	–	1.09	tr
$C_{31}H_{64}$ alkane (hentriacontane)	–	–	1.14	1
24-Methylene cholesterol[d]	–	–	1.18	tr
Campesterol[d]	–	–	1.19	1
Stigmasterol[d]	0.17	–	1.21	1
β-Sitosterol[d]	0.17	0.79	1.23	2
β-Amyrin[d]	0.28	0.83	1.23	1
Δ^5-Avenasterol[d]	–	–	1.23	tr
α-Amyrin[d]	0.28	0.83	1.24	5
Ψ-Taraxasterol[d,e]	–	–	1.25	2
β-Amyrin acetate	0.61	0.91	1.25	11
α-Amyrin acetate	0.61	0.91	1.27	51
Ψ-Taraxasteryl acetate[e]	–	–	1.30	1
Oleanolic acid[d]	–	–	1.31	tr
Ursolic acid[d]	–	–	1.32	tr
β-Amyrin butyrate	0.73	0.91	1.34	1
α-Amyrin butyrate	0.73	0.91	1.36	2
β-Amyrin caproate	0.75	0.91	1.43	2
α-Amyrin caproate	0.75	0.91	1.44	8
β-Amyrin palmitate	0.79	0.91	1.79	tr
α-Amyrin palmitate	0.79	0.91	1.82	2
Triglycerides	0.57	0.87	2.08	tr

[a]TLC conditions: silica gel GHLF; solvent system A: hexane/ethyl ether/acetic acid (80:20:1); solvent system B: methylene chloride/methanol/acetic acid (90:10:1).

[b]GLC conditions: see text.

[c]Tr (trace) <0.5%.

[d]Subjected to GLC and GC-MS analysis as the corresponding trimethylsilyl derivatives.

[e]Tentative compound identifications based on MS and RR_t data (authentic standards not available).

eter using $CDCl_3$ as solvent and TMS as internal standard. Rubber molecular weights were determined by gel permeation chromatography (GPC) on a Hewlett-Packard 1081 B liquid chromatograph equipped with a refractive index detector and a 3388 A integrator system. A Micro Pak TSK GMH6 column was used for GPC, with polystyrene and *cis*-polyisoprene standards, using tetrahydrofuran as solvent.

Portions of the refined hexane extracts were saponified with 0.5 N ethanolic KOH using standard procedures (25). Samples of α- and β-amyrin acetate, butyrate, caproate, and palmitate were prepared by separately esterifying the free triterpene alcohols with acetic, *n*-butyric, and *n*-hexanoic anhydrides (using pyridine as catalyst) and palmityl chloride, respectively.

Using these procedures, over 90% of the constituents of both the crude and refined hexane extracts could be identified and quantitated, and the results are summarized in Tables I and II.

RESULTS AND DISCUSSION

The crude hexane extract of *A. speciosa* typically comprises 3.5–7% of the dry weight of the plant material. Pigments account for ca. 12% of the hexane extract, whereas low molecular weight natural rubber comprises ca. 2% of the extract (Table I). Proton NMR confirmed the rubber of *A. speciosa* to be *cis*-1,4-polyisoprene, as in *A. syriaca*, *Hevea* and guayule (1). Unfortunately, the rubber of *A. speciosa* is of too low a molecular weight and is present in too small a quantity to directly substitute for *Hevea* or guayule rubbers, although it may find use in elastomeric applications.

Only very small amounts of fatty acids, alcohols, hydrocarbons (alkanes and squalene), monoglycerides and phytosterols were found in the hexane extracts (Table II). Although triglycerides are not readily quantitated by capillary GLC methods (26), TLC with triolein as standard established that only traces of triglycerides were present in these extracts (3, 6, 27). TLC analysis also confirmed the absence of significant amounts of cardiac glycosides from the hexane extract.

The major portion of the hexane extract (ca. 86%) was found to consist of derivatives of α- and β-amyrin and related pentacyclic triterpenes. Over 60% of the decolorized, rubber-free hexane extract was found to consist of α- and β-amyrin acetates, present in a ratio of ca. 5:1. Smaller amounts of the corresponding butyrate, caproate, and palmitate esters of these triterpenes were found in roughly the same ratio of α- to β-derivatives (Table II). Overall, triterpene esters account for ca. 78% of the refined hexane extract of *A. speciosa*. Unequivocal proof of the identities of these esters was obtained by chromatographic (TLC, GLC) comparison of the *A. speciosa* extractables with the corresponding semisynthetic esters prepared following saponification.

Sterols account for ca. 5% of the refined hexane extract and pentacyclic triterpenoids account for ca. 86%. Thus, over 90% of the refined hexane extract of *A. speciosa* consists of sterols and triterpenoids.

It is noteworthy that no tetracyclic triterpenoids were found in the nonpolar extracts of *A. speciosa*. This contrasts with the hexane extractables of *Euphorbia lathyris*, another hydrocarbon-producing plant considered by some to be a candidate for domestication and cultivation (4, 8, 9, 28, 29). The nonpolar extracts of *E. lathyris* were found to contain ca. 85% of both tetracyclic and pentacyclic triterpenoids, many of which were present in esterified forms (4, 8, 9, 28, 29). However, it is interesting to note that although the detailed composition of the nonpolar extractables from *A. speciosa* and *E. lathyris* differ with respect to specific compounds, the percentage of compound classes represented appear to be very comparable in both species.

Preliminary studies using TLC and GLC have shown that the hexane extractables of *Calotropis procera* and other milkweeds such as *A. syriaca* and *A. latifolia* are qualitatively similar to those of *A. speciosa*.

As in the case of *E. lathyris* (4, 8, 9), the chemical structures and physical properties of the nonpolar extractables of *Asclepias* species (and *Calotropis procera*) make them unsuitable for direct use as conventional fuels without additional processing via catalytic conversion. However, the refined *Asclepias* nonpolar extractables may find more direct use as higher value industrial raw materials (3, 6, 7). Such applications include uses as plasticizers, as processing aids for rubber and in coatings and polish formulations (3, 6, 7). In view of these higher value applications, *Asclepias* species appear to be worthy of further crop development. In addition, advances in extraction and processing technology, and improved yields and agronomic characteristics obtained through selection and breeding, may make *Asclepias* species even more attractive as crops in the future.

Although we have already identified the main constituents of the methanol extracts of *A. speciosa* to be *myo*-inositol and sucrose (30), work is continuing to identify and quantitate other polar constituents of toxicological and/or economic importance, such as the cardiac glycosides known to occur in milkweeds (31–33). The extraction and processing of these polar constituents may yield products which will give added value to *A. speciosa* as a crop. In addition, economical detoxification of the bagasse (marc or residue) so that it can be used as an animal feed is an important consideration for the commercial development of *A. speciosa*, and work is likewise continuing in this area.

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Fatty Acid and Sterol Compositions of Malagasy Tamarind Kernel Oils

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ABSTRACT

The oil contents of six samples of Malagasy tamarind (*Tamarindus indica* L.) kernel were determined by hexane extraction (6.0–6.4%) and chloroform/methanol extraction (7.4–9.0%). The protein contents were very low (trace–0.1%). Investigation by gas liquid chromatography revealed 15 fatty acids, mainly palmitic (14–20%), stearic (6–7%), oleic (15–27%), linoleic (36–49%), arachidic (2–4%), behenic (3–5%) and lignoceric (3–8%) acids. Testing for the sterol fraction enabled seven sterols to be separated and quantitatively analyzed by gas liquid chromatography. The main sterols were β-sitosterol (66–72%), campesterol (16–19%) and stigmasterol (11–14%).

INTRODUCTION

Tamarindus indica L. (Caesalpiniaceae) is cultivated in many parts of the world: India, Florida, Egypt, Sudan, Formosa, Southeast Asian countries (1) and Madagascar. This tree is probably indigenous to this last country (2) and possibly to some parts of South India (3). The fruit is a flat pod which on ripening gives an edible pulp. Brown and flat seeds are decorticated and powdered kernel, commercially known as tamarind kernel powder (TKP), is largely used in the textile industry (1) but also in food industries because of the occurrence of a characteristic tamarind seed polysaccharide which is able to form jellies with sugar concentrates over a wide pH range (1). The TKP contains 6–8% oil, which has been investigated for its fatty acid composition by several authors (4–10). Large differences in results have been observed in the fatty acid composition of the oil and in the protein content of the TKP between the Indian (4, 5, 8–10) and the Egyptian (7) products.

In Madagascar, tamarind trees grow almost all along the western part of the island. Fruit pulp is consumed by the population but the seeds are lost. Since this unused TKP may represent a potential agroeconomical interest for Madagascar, we have investigated the chemical characteristics of six seed samples of tamarind trees growing in various parts of the island. The fatty acid and sterol compositions were also analyzed using gas liquid chromatography (GLC) and combined GLC-mass spectrometry (GLC-MS).

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EXPERIMENTAL PROCEDURES

Materials

Tamarind fruits were collected during 1981 in six different areas of Madagascar island (Ambanja, Ambato-Boeni, Ambila, Majunga, Miandrivazo and Tulear). The tamarind kernel powders were prepared in the following manner: the seeds were washed with water in order to free them from attached pulp. The seeds were treated with concentrated sulfuric acid (36 N) and warmed at 80–100 C, for 30 min. The testae were dissolved slowly and the remaining white kernels were ground into powder.

Physicochemical Methods

Moisture, total proteins, ash and unsaponifiable matter contents were determined according to NFT 60-201; NFT 18-100; NFT 60-209 and NFT 60-205 Norms (11), respectively. Oils were extracted either with hexane, or with chloroform/methanol (2:1, v/v) in a Soxhlet apparatus for 10 hr by a modified Folch method (12). The solvent was removed using a rotary vacuum evaporator.

Analysis of Fatty Acid Composition by GLC

Fatty acid methyl esters were prepared by saponification of oils and acid-catalyzed methylation using BF_3-CH_3OH (10%, Fluka, Switzerland) according to NFT 60-233 Norm (11). Purification of the methyl esters was done as previously described (13). Commercial saturated even-numbered methyl esters (Fluka), unsaturated and polyunsaturated (Sigma, St. Louis, MO) were used as standards for the identification of methyl esters together with vegetable oils (e.g., peanut, olive, sunflower).

A Girdel 30 gas chromatograph (Girdel, France) equipped with a flame ionization detector was used for the